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THE ECOLOGY OF *CHONDRUS CRISPUS* AT PLYMOUTH, MASSACHUSETTS. III. EFFECT OF ELEVATED TEMPERATURE ON GROWTH AND SURVIVAL[1]

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The specific relationship between temperature and the responses of attached marine algae has had limited attention. Of particular current interest is the effect of elevated temperatures on growth, reproduction, and survival of populations of algae at locations where additions of heat are proposed in the coastal environment. Ignatiades and Smayda (1970), Ott (1966) and Provasoli (1963) found that temperatures of 25°–30° C were frequently above optimum for growth of cold water marine algae in culture. Adey (1970) showed that growth of boreal subarctic *Lithothamniae* declined with temperatures above 10°–15° C. Studies by Ehrke (1931) of respiration and photosynthetic rates with *Fucus, Enteromorpha,* and *Delesseria,* and by Kanwisher (1966), Mathieson and Burns (1971), and Newell and Pye (1968) with *Chondrus* demonstrate that thermal injury to the metabolism of these species occurs at temperatures above 26° C. Ring (1970) noted that holdfasts of *Chondrus* died in culture within two days at 29° C, but the erect shoots survived for two months at this temperature. Growth of the erect shoots was better, however at 10° than at 15° C. Burns and Mathieson (1972) found that sporeling growth increased with an increase in temperature up to 19° C. In comparison, Newton, Devonald, and Jones (1957) failed to find a respiratory maximum for *Chondrus* even at 35° C.

These data do not allow for confident predictions, even approximate, on the effect of thermal additions on populations of attached marine algae. A nuclear thermoelectric generating plant is under development at Rocky Point, Plymouth, Massachusetts (Prince and Kingsbury, 1973b). Its thermal discharge is located in the midst of one of the principal commercial beds of *Chondrus crispus* on the western Atlantic coast. The purpose of this study was to culture *Chondrus* under controlled conditions at a growth rate approximating that in nature and to determine the effects of elevated and depressed temperatures on growth and survival. We have reported the growth and reproductive responses of the natural population at Plymouth in the other papers of this study (Prince and Kingsbury, 1973a, 1973b).

Materials and Methods

Three series of culture experiments were conducted between January and December, 1970, to establish normal growth curves, to identify optimum conditions,

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and to perfect techniques of culture, experimentation, and examination. These experiments also yielded information on growth of sporelings at temperatures and light conditions above and below optimum. In this work it became necessary to move culture facilities from Ithaca, New York, to New London, Connecticut. Seawater for culture media was obtained from several locations and handled in different ways. Lighting conditions and measurements of light intensity also varied as techniques were refined. For these reasons, results of the three series are not exactly comparable. We describe here methods and results for the third series of experiments, the most refined, with comments on the preceding two series only where results amplify those of the third series. Following this work, we did additional sets of experiments to look more closely at specific lethal or deleterious characteristics of elevated temperatures.

Culture chambers were illuminated with dual 40 watt cool white fluorescent tubes (Westinghouse F40CW). Intensity was regulated by varying the distance of the cultures from the tubes, covering one of the tubes, or interposing a plastic screen filter. Intensity was determined in m watts/cm² (Yellow Springs Radiometer, No. 65, probe 6551 set on a large heat sink, the whole covered by a petri dish to give readings equivalent to those inside a culture flask). Several readings were taken on each shelf and averaged.

Temperature in the culture cubicles was monitored by bimetallic, Fahrenheit recorders (Bachrach Tempscribe) checked by a centrigrade mercury thermometer. Temperatures were held to within ±0.6° C of desired, with a diurnal fluctuation of 1.0° C as the lights went on and off. Most experiments were conducted under a light regimen of 16 hours light, 8 hours dark (16:*8*) ; some at 10:*14*.

Culture medium was constructed with natural seawater, filtered (Whatman No. 1), and enriched with nitrate, phosphate, soil extract, and a vitamin and trace metal solution based upon Guillard's medium "f" (Guillard and Ryther, 1962) at half strength. The composition of the culture medium was as follows: KNO_3 72.2 mg, KH_2PO_4 8.0 mg, (the latter two based upon Iwasaki, 1961), $CuSO_4 \cdot 5H_2O$ 0.01 mg, $ZnSO_4 \cdot 7H_2O$ 0.022 mg, $CoCl_2 \cdot 6H_2O$ 0.01 mg, $MnCl_2 \cdot 4H_2O$ 0.18 mg, $NaMoO_4 \cdot 2H_2O$ 0.006 mg, Fe (as Ferric sequestrine, 13% iron; Geigy Indust. Chemicals, New York) 5 mg, Thiamine 0.2 mg, B_{12} 1 μg, Folic Acid 1 μg, Biotin 1 μg, Riboflavin 0.25 μg (based upon Provasoli, 1964), soil extract 50 ml, seawater (aged at least 2 months) to one liter. The seawater used in the third series of experiments was collected at the University of Connecticut's Marine Station at Noank in March, 1970, and was aged seven months before use. Its salinity varied between 29 and 31‰; that of the medium made from it was 1‰ less. The pH of the medium varied between 8.1–8.3. All media were sterilized before use by autoclaving. Precipitation problems were minimized by using aged seawater, and very slow cooling.

Reproductive plants were collected at Rocky Point and Manomet Point (Prince and Kingsbury, 1973b) in seawater, iced during transport to Connecticut, and refrigerated (0.5°–3.0° C) overnight. The following morning, tips with apparently ripe carposporic or tetrasporic sori were excised from the cleanest and least epiphytized plants, washed with jets of sterile seawater at 12° C or scraped with a coarse artist's brush and forceps, and placed in petri dishes of sterile seawater at 11.7° C. Spore release sometimes occurred on the same day as preparation of the tips, pos-

sibly in response to temperature variations in handling, but was more usual on the second to fourth days. Tips not discharging by the fourth day were discarded. The discharged spores form a pink-red spot under the tip and must be gathered within 2–4 hours after release or they become attached too firmly to each other and the substrate for removal. Fresh spore masses were picked up with a Pasteur pipette, sprayed over a glass side in a petri dish of sterile seawater, and allowed to settle for 0.5 hours after which the spores were firmly attached to the slide. These manipulations were carried out in a room at 21° C. The temperature of the seawater medium rose during the period of manipulation from 12° to approximately 18° C before the cultures were placed in the cubicles.

Growth of sporelings was recorded as a function of the diameter of the holdfast. Three slides were examined per spore type (tetraspore or carpospore), experimental condition, and age, and 15–40 holdfasts were measured per slide. Growth curves were fitted by inspection to a semi-log plot of sporeling area *vs.* time. Doubling of area per day (K) was calculated:

$$K = \ln \frac{A_2}{A_1}\left[\frac{1}{(T_2 - T_1) \ln 2}\right]$$

where A_2 and A_1, the areas of the sporelings at times T_2 and T_1 respectively, were selected within the interval of the logarithmic phase of growth.

For examination, aqueous mounts were made of the experimental slides directly, using seawater at the same temperature as the experimental conditions (except that water at 11.7° C was used for cultures maintained at 4.4° C to avoid coverglass fogging). Measurements, at 100 or 430 magnification as necessary, were accomplished within 15–30 minutes per slide in a 20° C room. Slides were restored to culture conditions immediately after examination.

By experimental series III techniques had evolved to the point where contamination of cultures was a minor problem. Diatoms were the most frequent contaminants. Slides containing large numbers of sporelings (*ca.* 200) were occasionally washed with a jet of sterile water to remove any percipitated medium or diatoms. Others were handled only if necessary. In those few cultures which developed persistent contamination with diatoms, it could be kept under control by weekly washing with sterile water and an artist's brush. This treatment may have removed dead or poorly attached spores of *Chondrus,* but had no apparent effect on established germlings. In the initial series of experiments, germanium dioxide (10 mg l) was used to eliminate diatom contamination (Lewin, 1966), without apparent effect on the morphology or growth rate of young *Chondrus* plants.

The exact time at which an organized, multicellular holdfast or erect shoot dies is difficult or impossible to determine. Mortality of spores is easier to recognize as an event in time. Experiments on lethal or injurious effects of elevated temperatures therefore were conducted with freshly released, apparently healthy spores. We have described the morphology and cytology of normal spores and spore germination elsewhere (Prince and Kingsbury, 1973a). Death of spores is characterized most conspicuously by loss of color and rhodoplast organization.

In the first set of mortality experiments, cultures were initiated in the same manner as described above. After a half hour in the 20° C preparation room to

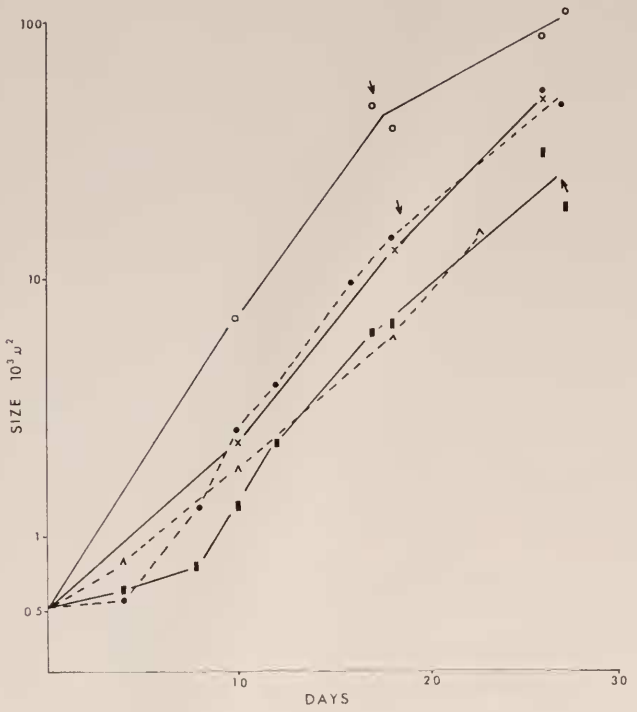
allow settling and attachment, cultures were transferred to culture rooms and grown at 0.35 m water/cm², 16:*8* photoperiod, at 11.7°, 21.1°, and 23.9° C. After four days, 10–15 separate fields were counted for each culture, and at least 3 cultures were counted per spore type and temperature. Proportion of dead to living spores was recorded and analyzed statistically. In the second set of these experiments, intended to simulate the effects of sudden exposure to a thermal plume, handling was the same except that the spores were given a brief period of thermal elevation while held in pipettes before being sprayed on the culture slides. Thermal "shock" periods of 0.5, 1.0, or 6.0 minutes were administered by immersion of the sealed pipette tip in a water bath. After the shock period, spores were sprayed over a slide in media at 21° C, held for 0.5 hours at this temperature, then cultured at 11.7° C, 0.35 m watts/cm², and 16:*8* light. The interposition of 0.5 hours at 21° C was intended to reproduce the effect of thermal effluents in which subsequent cooling would not be instantaneous. Controls were subjected to the same intermediate warm period. Condition of spores was determined four days after the "shock" treatment, recorded and analyzed as above. A third set of experiments was conducted with fully developed plants held at 26.7° C for an extended period, other conditions similar to the above.

Results

Chondrus spores germinate and grow well in culture at temperatures similar to those found in the field. An initial lag phase (Fig. 1A and B) is followed by a logarithmic phase of growth. Initiation of the erect shoot from the original sporeling holdfast (Prince and Kingsbury, 1973a) is accompanied by diminution in the growth rate.

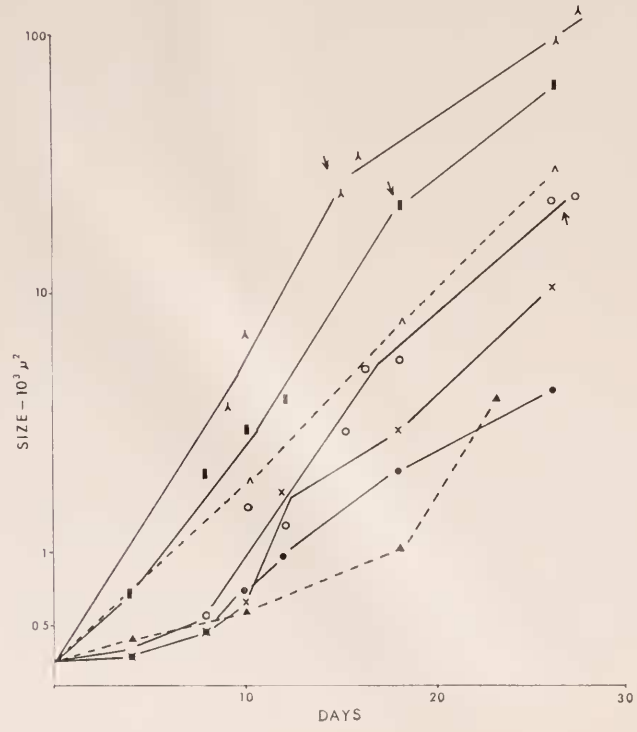
During the lag phase, single-celled and few-celled sporelings predominated. Little increase in diameter of the sporeling accompanied the first divisions; subsequent increase was marked and regular. The presence and extent of the lag phase appears to be related to spore type, light intensity, and particularly temperature. We do not have enough points to establish the duration of a lag phase under the most rapid development characteristic of optimum conditions.

The termination of the logarithmic phase of growth of the sporeling holdfast coincided with organization and appearance of the erect shoot from it (arrows, Fig. 1A and B). In this reorganization of growth pattern, major meristematic activity is transferred from the rim of the holdfast to the tip of the newly forming shoot. In experiments of the earlier series carried to day 51, 92–100% of the carpospore-derived sporelings produced erect shoots, but only 72–87% of the tetraspore-derived holdfasts had done so under the same cultural conditions. Initiation of erect shoots by sporelings under equivalent conditions appeared to be primarily a function of size of the holdfast. Sporelings grown under optimal conditions produced erect shoots significantly earlier than did sporelings grown under suboptimal conditions. For example, in one experiment, tetraspore-derived holdfasts grown at 11.7° C required an average 26 days before erect shoot formation; those at 21.1° C formed erect shoots on the 18th day. The threshold size of holdfasts in these experiments appeared to be about 10×10^3 μ^2. Under suboptimal photoperiod (10:*14* instead of 16:*8*), however, tetraspore- and carpospore-derived



°C	mWatts / cm²	light
o 21.1	0.35	16/8
x 21.1	0.35	10/14
• 11.7	0.98	16/8
▮ 11.7	0.35	"
^ 23.9	0.35	"

A – Carpaspores



°C	mWatts / cm²	light
^ 21.1	1.18	16/8
▮ 21.1	0.35	"
^ 21.1	0.35	10/14
o 11.7	0.98	16/8
x 11.7	0.35	"
• 11.7	0.13	"
▲ 23.9	0.35	"

B – Tetraspores

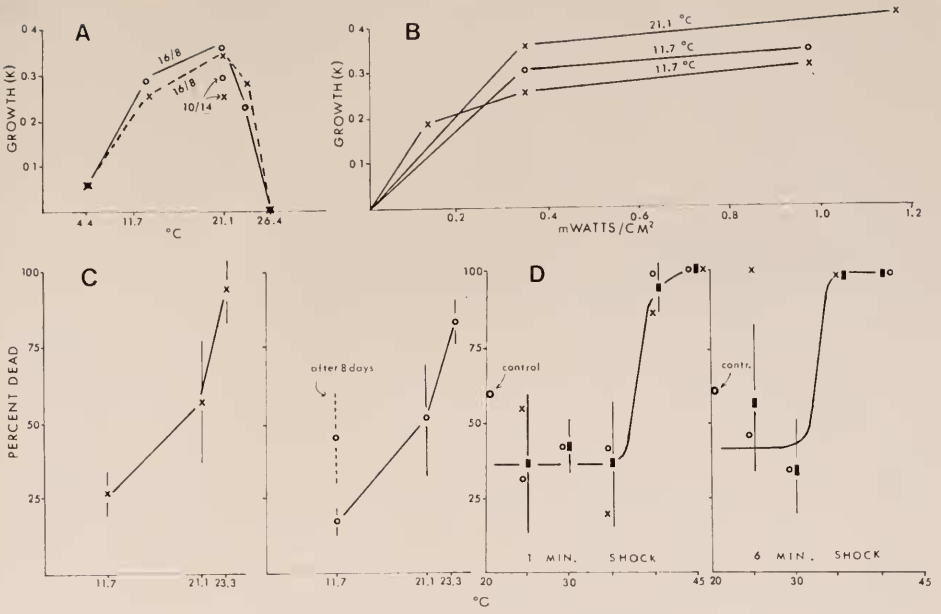


FIGURE 2. (A) Effect of temperature on growth rate of germlings. Curves are for light regimen 16:*8;* two additional points for 10:*14* also shown; in all figures: x, tetraspores or tetraspore-derived germlings; o, carpospores or carpospore-derived germlings; (B) effect of light intensity on growth rate of germlings at two temperatures; (C) mortality of spores after 4 days at elevated temperatures; (D) mortality of spores after brief shock treatment with elevated temperatures; curves by inspection.

sporelings which exceeded this size had not formed erect shoots by the end of the experiment.

Under our experimental conditions, temperature presented the greatest effect on growth during the logarithmic phase (Fig. 1A and B). Some experiments in the earlier series (not graphed) were conducted at 4.4° C. At that temperature, germination and growth of tetraspore- and carpospore-derived sporelings were severely inhibited. After 19 days the cultures still consisted mainly of undivided spores; only a few 2–4 celled sporelings were present. After 25 days a few small holdfasts had developed, but undivided spores were still in the majority. At the other extreme (also not graphed), cultures maintained at 26.7° C displayed signs of injury and death, and did not grow. All carpospores were dead within 4 days; all tetraspores within 4–10 days in experimental series III. In experimental series II a few carpospores survived or developed into few-celled sporelings at this temperature. These sporelings were characterized by thick walled cells and were poorly attached to the culture slide.

The relationship of growth rate to temperatures between these high and low extremes is presented in Figure 2A. The curve ascends to an optimum value

FIGURE 1. Growth curves for germlings of *Chondrus crispus* at specified conditions of light and temperature. Germlings initiated from carpospores (A) or tetraspores (B). Arrows indicate points at which erect shoots were initiated for those cultures which produced them.

(of those employed) at 21.1° C, then drops off sharply. It is similar for sporelings derived from tetraspores and carpospores, although carpospore-sporelings regularly exhibited more rapid growth. For both types of sporelings the increase in growth rate per degree centigrade was greater between 4.4 and 11.7° C than between 11.7 and 21.1° C. Burns and Mathieson (1972) present results to the contrary; however, their experiments did not take into consideration the effect of a lag phase on growth.

At 23.9° C the growth rate was slightly less than that at 11.7° C. Furthermore, the sporelings grown at 23.9° C displayed morphological and cytological abnormalities. In these cultures some spores (whether tetraspore or carpospore) remained undivided, became highly vacuolated, and increased in size. This type of cell was also frequently observed in cultures at 26.7° C; it appeared incapable of cell division. Holdfasts that developed in cultures at 23.9° C were often irregular in outline and displayed an astral pattern of coloration. Color was confined to sectors extending from the center of the holdfast to the periphery. In contrast, holdfasts grown at 21.1° C were usually circular in outline, and only the cells of the rim, the location of active cell division, were colorless (Prince and Kingsbury, 1973a). Number of plants measured (1401 at 21.1° C and 429 at 23.9° C) and constancy of results in experimental series III support the reality of a sharp break in the growth curve between these two temperatures.

Comparison of the effect of light intensity on growth was made with tetraspore sporelings at two temperatures and with carpospore sporelings at a single temperature (Fig. 2B). The two types of sporelings responded similarly. Three intensities were employed. In all cases, greater intensity produced greater growth, but the effect was nearly level at intensities of 0.35 m watts/cm² and above. Photoperiod displayed a definite effect where temperature and intensity were held constant (Fig. 1A and B). At optimum conditions of 21.1° C and 0.35 m watts/cm², the growth rate of tetraspore and carpospore sporelings declined 31% and 19% respectively, with a 38% reduction in light duration (10:*14 vs.* 16:*8*). Photoperiod of 16:*8* is approximately equivalent to that of the summer solstice at the latitude of Plymouth.

At optimum temperatures, the death of a spore is readily discernible and takes place over a brief period of time. Appreciable mortality occurred in all cultures at all temperatures, and has been found by others (Burns and Mathieson, 1972). Above this background level, however, effect of elevated temperature on spore mortality was marked (Fig. 2C). Spore mortality was assessed in this representation as a function of total spores and sporelings in cultures grown at the three intermediate temperatures after four days. For each spore type and temperature condition, 600–1200 spores were evaluated. Mortality increased with increase in temperature from 17–26% at 11.7° C, to 84–94% at 23.9° C, (to 100% at 26.7° C from earlier results). Carpospores showed a lower mortality at all temperatures than did tetraspores. We hypothesized that incipient mortality might not become apparent at 11.7° C within four days because of the "refrigeration effect" of this low temperature. Therefore, carpospores were reexamined after 8 days in the cultures at 11.7° C. Carpospore mortality had increased from approximately 15% to 45% in the intervening 4 day period. Carpospores tend to remain intact longer after death than do tetraspores which apparently have thinner and more

fragile cell walls. Although spore mortality may be considerable (to 60%, depending on circumstances) at temperatures up to and including 21.1° C, mortality is closely related to increasing temperatures above that point, and rapidly approaches 100%.

Brief shock treatments at elevated temperatures present similar results. Exposure of 30 seconds at 35° C yielded only 3.5% mortality. At 45° C the same treatment yielded 100% mortality. Results of shock treatments at other temperatures and durations are presented in Figure 2D. In each case, a range of appreciable mortality with large standard deviations (including controls) occurs up to a certain temperature, and then increases rapidly with temperature. The temperature at which "entrainment" of mortality occurs is distinctly higher for the briefer period of exposure.

Cultures from tetraspores and carpospores, 187 days old and bearing erect shoots averaging 7.0 mm and 9.1 mm, respectively, were transferred from 11.7° to 26.7° C and held at the elevated temperature with periodic renewal of media for 35 days. After 16 days erect shoots of the tetraspore culture had assumed a yellowish-red color while the carpospore culture had turned from maroon to brick red. After 35 days, 41 tetraspore-derived individuals were $\frac{1}{2}$–$\frac{3}{4}$ dead (white). Several others were entirely dead (erect shoot and holdfast). The population macroscopically was light red. In the parallel cultures of carpospore-derived individuals, none were entirely or mostly dead, and the color remained a brick red.

Discussion

Our results suggest that *Chondrus crispus* will benefit in increased growth from increased temperatures through 21.1° C at Plymouth. At temperatures above that, growth drops sharply, and abnormalities increase. Temperatures above 21.1° C also have a profoundly deleterious effect on reproduction by causing death of spores if prolonged. Above 30° C even brief exposures will result in spore mortality.

Nuclear thermal electric generators are generally designed to spread a heated effluent onto the surface of the receiving waters to maximize the release of heat to the atmosphere. The zone of maximum heat from a generating station can be expected to lie primarily above populations of *Chondrus*. Any warming of the waters, particularly in the summer, brought about by operation of a power plant should result in increased growth of *Chondrus*, epiphytic algal species (Prince and Kingsbury, 1973b), as well as other eurythermal species. Increased competition for available substrate, nutrients, and light energy should result. Excessive epiphytism of *Chondrus* decreases the commercial value of the crop. If climatological or operational excursions result in impaction of maximally heated effluent against the *Chondrus* beds, however, a disaster may result.

Summary

Chondrus crispus was cultured under conditions of light intensity, photoperiod, mineral nutrition, and temperature approaching or slightly exceeding those to be expected for a subtidal population at Plymouth. Of these, only temperature beyond a certain point brought reduced growth or mortality. The growth curve breaks sharply between 21.1° and 23.9° C, and morphological abnormalities appear.

Cultures do not grow at all at 26.° C. As temperatures are elevated above 21.1° C, healthy spores die in culture in increasing numbers. Healthy spores exhibit mortality approching 100% when exposed to temperatures above 35–40° C, even for periods as brief as one minute, as might be encountered during entrainment in a thermal plume.

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